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EVALUATION OF *TALAROMYCES FLAVUS* AND *TRICHODERMA HARZIANUM* IN BIOLOGICAL CONTROL OF SUGAR BEET DAMPING-OFF DISEASE IN THE GREENHOUSE AND FIELD CONDITIONS

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ABSTRACT

Antagonistic effects of *Trichoderma harzianum* and *Talaromyces flavus* on sugar beet damping-off in the greenhouse and field were investigated. Antagonist fungi, obtained from Karaj sugar beet fields, were used in this study. Based on the results of greenhouse experiments, four most effective isolates (T.F.K.1- T.F.K.3- T.H.K.1 and T.H.K.2) were selected for the field studies conducted during 2008 and 2009. Field results indicated that in all intervals, treatments containing antagonistic fungi were superior to carboxin-thiram and control in terms of number of healthy seedlings. Among treatments, those containing T.F.K.3 had the maximum number of healthy seedlings. However, there was no significant difference between treatments containing T.F.K.1 and T.H.K.1 in terms of number of healthy seedlings and the yield. In addition, a significant increase was observed in the total yield for all antagonistic treatments compared to carboxin-thiram and control.

KEYWORDS: Biological Control, Seedling Damping, off Disease, Sugar Beet, *Talaromyces flavus*, *Trichoderma harzianum*

INTRODUCTION

Sugar beet (*Betae vulgaris*) is one of the most important crops which is presently cultivated in many countries around the world including Iran. The importance of this crop is due to its various use including a source of human and animal nutrition. Acreage of sugar beet fields in Iran is about 200000 hectares which is a considerable number, but the yield of this crop is low compared to other countries. Existence of different diseases is a major cause of low yield for this crop plant (Babai-Ahary *et al*., 2004).

Sugar beet damping-off disease is one of the most common diseases of this crop in Iran (Babai-Ahary *et al.,* 2004) and in the world (Nakayama *et al.,* 1999; Luterbacher *et al.,* 2005). Seed treatment with chemical fungicides is the most common method for combating this disease. Indiscriminate use of chemicals in the agriculture is an issue which has created severe health and environmental problems and has recently received serious criticism from environmental organizations. In addition to the above problems, the high cost and resistance of pathogens to chemical pesticides should be taken to the account. Considering the above-mentioned problems, it is essential to look for non-chemical combating methods such as biological control. Antagonistic fungi and bacteria have recently been used for controlling diseases and promoting growth characteristics in different plants (Ardakani *et al*., 2010; Naraghi *et al*., 2010a, b and c; Jorjani *et al*., 2011; Naraghi *et al*., 2012a and b; Gerami *et al*., 2013; Mansouri *et al*., 2013). Application of these antagonists has no significant costs in addition to having no risks for the environment and non-target organisms (Gray and Gerik, 1998; Bardin *et al.,* 2004; Heydari *et al.,* 2005; Abo-Elnaga, 2006; Sadeghi *et al.,* 2009).

In a previous study, Moussa (2002) indicated that sugar beet seedlings are usually attacked by pathogenic fungi such as Phytophthora, Phoma, Pythium, Aphanomyces, Rhizoctonia, Sclerotium, Fusarium and Polymyxa. Through a survey on the causes of sugar beet damping-off disease in Wyoming state during 1992 to 1994, Gray (1995) could isolate *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium* sp. from the infected sugar beet seedlings in all three years. However, *Phytophthora drechsleri*, *Phoma betae*, *Aphanomyces cochlioides* and *Rhizopus* sp. were present only in the first year of the study.

Numerous studies have also been conducted in biological control of these diseases through using antagonistic fungi (Cliquet and Scheffer, 1997) and bacteria (Shah-Smith and Burns, 1997) which have led to a decrease in sugar beet damping-off disease. For example Abada (1994) indicated that application of *T. harzianum* considerably reduced sugar beet root rot and damping off in the greenhouse and field and also increased the root weight in the field. Results of a field experiments conducted by Moussa (2002) for investigating the possibility of biological control of sugar beet, damping-off showed that *T. harzianum* was the most effective compared with other treatments including fungal and bacterial antagonists.

In another study, Ambrosino *et al.* (2004) reported that Trichoderma species which were commercially used in the market as the biocontrol agents and soil amendment significantly reduced the application of chemical compounds and their consequences (existence of its residues in foods). In this regard a highly effective biocontrol fungus, *T. harzianum* T22, which has shown significant antagonistic effects against many plant pathogenic fungi including *R. solani*, *Botrytis cinerea* and *P. ultimum* was studied for its mechanisms and the proteins of this strain with biotechnological potential was identified for industrial and commercial use. According to the results of the above-mentioned study, some of these proteins are generated through interaction of strain *T. harzianum* T22 with plant pathogenic fungi and their application can lead to the induction of systemic resistance in plant.

Ciliento *et al*. (2003) also showed that application of Trichoderma species in agriculture not only has a direct inhibitory effect on plant pathogenic fungi, but it also increases its tolerance to environmental stresses through improving the plant growth factors. Furthermore, El-Tarabily (2004) indicated that some yeasts have also controlled the seedling damping-off disease caused by *R. solani* as well as enhancing the host plant growth. Bardin *et al*. (2004) indicated that the effect of experimental treatments containing the strains of *R. leguninosarum* led to the significant decrease in the percentage of sugar beet seedling damping-off caused by *Pythium ultimum* compared to control. The results of studies in the field of biological control of sugar beet seedling damping-off *R. solani* disease in Iran through applying the isolates of *T. harzianum* fungus in the laboratory and greenhouse indicated that all isolates showed hyphal destruction of Rhizoctonia by contacting and wrapping around them (Shahiri Tabarestani, 2005; Shahraki *et al*., 2008). Moreover, the results of greenhouse investigation in this study indicated that seed treatment with isolate of *T. harzianum* and its addition to the soil can significantly reduce this disease.

The objectives of this study were to investigate the efficacy of *T. flavu*s and *T. harzianum* isolates in biological control of sugar beet seedling damping-off disease.

MATERIALS AND METHODS

Greenhouse Experiment

A greenhouse experiment was conducted and executed based on the number of pathogenic fungal isolates obtained from sugar beet fields in Karaj area with the following treatments:

1- Seed and soil without pathogen (healthy control). 2- Seed without pathogen and soil inoculated with isolate R.S.K.4 (infected control). 3- Seed without pathogen and soil inoculated with isolate R.S.K.5 (infected control). 4- Seed without pathogen and soil inoculated with isolate F.S.K.1 (infected control). 5- Seed without pathogen and soil inoculated with a mixture of pathogenic isolates (infected control). 6 to 9- Soil similar to the treatments 2 to 5, but seed treated with carboxin-thiram. 10 to 13- Soil similar to the treatments 2 to 5, but seed treated with isolate T.F.K.1. 14 to 17- Soil similar to the treatments 2 to 5, but seed treated with isolate T.F.K.2. 18 to 21 - Soil similar to the treatments 2 to 5, but seed treated with isolate T.F.K.3. 22 to 25- Soil similar to the treatments 2 to 5, but seed treated with isolate T.H.K.1. 26 to 29- Soil similar to the treatments 2 to 5, but seed treated with isolate T.H.K.2. 30 to 33- Soil similar to the treatments 2 to 5, but seed treated with isolate T.H.K.3.

Field Studies

After conducting the greenhouse experiment where three isolates of *Trichoderma harzianum* and three isolates of *Talaromyces flavus* were applied in order to reduce the sugar beet damping-off disease, and based on the obtained results, two isolates of *T. harzianum* and two isolates of *T. flavus* were selected for the field experiment. The experiment was conducted during two years, 2008 and 2009, using common sugar beet cultivar (Rasul) in a randomized complete block (RCB) design with 6 treatments and 4 replications. Treatments included: 1 to 4- Seeds treated with fungal antagonistic isolates, 5- Seeds treated with carboxin-thiram, 6- Seeds without any pathogen (healthy control). Evaluating the effect of test treatments on controlling the pathogenic fungus was done through determining the number of healthy seedlings and total yield. Data recording was done for calculating the average number of healthy seedlings 20, 30, 40 and 60 days after planting. For preparation of *T. flavus* and *T. harzianum*, the proliferation of mentioned fungi was carried out on the rice bran bed (Naraghi *et al.*, 2007).

Statistical Analysis of Data

The data were subjected to analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test by MS TAT C statistical software (Johannes Kepler University of Linz, Austria). The level of significance was determined in different treatments at 1% probability.

RESULTS

Preparation of Fungal Antagonists

Three isolates of *T. flavus* (TF.K.1 - TF.K.2 and TF.K.3) and three isolates of *T. harzianum* (TH.K.1 - TH.K.2 and TH.K.3) were isolated from the Karaj fields soil and identified as the above-mentioned fingi.

Isolation of the Pathogenic Fungi from the Soil of Sugar Beet Fields

At this stage, two isolates of *Rhizoctonia solani* and one isolate of *Fusarium proliferatum* were separated from the soil of sugar beet fields obtained in Karaj area and were encoded as R.S.K.4 – R.S.K.5 and F.S.K.1.

Pathogenicity Test

Pathogenicity test was carried out for all pathogenic fungal isolates and results showed that application of all isolates resulted in the appearance of damping-off disease on sugar beet seedlings. The fungal pathogens were also reisolated from the seedlings showing the disease symptoms.

Comparison of Treatments Affected by *T. flavus* and *T. harzianum* in Greenhouse Conditions

The results of this experiment indicated that the average number of healthy seedlings in all treatments affected by isolates *T. flavus* and *T. harzianum* was higher than those without these isolates 15, 30 and 45 days after sowing.

Meanwhile, in the case of disease-causing *Rizoctonia solani* isolates (R.S.K.5 and R.S.K.4) and combination of all pathogenic isolates, the treatments affected by *T. flavus* isolates were more effective than those affected by *T. harzianum* isolates. While, in the presence of Fusarium pathogenic isolate (F.S.K.1) the average number of healthy seedlings in treatments affected by *T. harzianum* was higher than the treatments affected by *T. flavus* (Table 1).

Furthermore, about the effect of antagonistic isolates on disease reduction, the results indicated that among the isolates of *T. flavus* isolates T.F.K.1 and T.F.K.3 showed higher efficacy than T.F.K.2. However, among the isolates of *T. harzianum*, T.H.K.1 and T.H.K.2 were more effective than T.H.K.3 (Table 1).

Field Studies

The results of field experiments in Karaj during 2008 showed that differences among treatments in the number of healthy seedlings were statistically significant at 1% of probability level 20, 30, 40 and 60 days after planting. Results also indicated that the maximum average number of healthy seedlings was belonged to the treatments T.H.K.1, T.H.K.2 and T.F.K.3, respectively (Table 2). Moreover, the results also showed that there were significant difference among different treatments at 1% of probability level in terms of total yield at the end of growth period. According to the results, all treatments affected by antagonistic fungi had higher yield in a range of 42,083 -54167 kg/ha compared to the control (37292 kg/ha) (Table 2).

The results of Karaj field experiment during the year 2009 showed that the number of healthy seedlings (stand) in different treatments was significantly different at 1% probability level 20, 30, 40 and 60 days after planting. According to the results the maximum average number of healthy seedlings in all periods of time belonged to the treatment T.F.K.3 (Table 3). Results also showed that all treatments affected by antagonistic fugal isolates had significant higher yield in a range of 36146 -63750 kg/ha compared to the control (26667 kg/ha) (Table 3).

In order to classify six treatments applied in studies, the combined analysis was performed on data regardless of crop year. Results of statistical analysis showed that the difference among treatments were significant at 1% of probability level. At all intervals including 20, 30, 40 and 60 days after planting, all treatments influenced by antagonistic isolates were superior to the control and Carboxin-thiram treatment in terms of average number of healthy seedling. Among the antagonistic treatments, the treatment contained (T.F.K.3) had the maximum average number of healthy seedlings. The maximum rate of total yield was also belonged to the above-mentioned treatment. However, there was no significant difference between the treatments affected by T.F.K.1 and T.H.K.1 in term of average number of healthy seedlings (40 and 60 days after planting) and total yield (Table 4).

DISCUSSIONS

Overall results of this study indicate that it may be possible to promote health and growth of sugar beet using *Talaromyces* and *Trichoderma* fungal antagonists. These fungal antagonists were capable of both disease suppression and promotion of growth and yield of sugar beet in the greenhouse as well as field conditions.

Trichoderma and *Talaromyces* have previously been used in the biological control of several plant diseases including cotton seedling damping-off, cucumber wilt, potato wilt and tomato wilt diseases (Howell, 2002; Naraghi *et al*., 2010a, b and c). Seedling damping-off which is one of the most important diseases of sugar

beet around the world has recently been controlled by the application of different microbial antagonists including fungi and bacteria (Shahiri Tabarestani, 2005; Shahraki *et al*., 2008; Jorjani *et al*., 2011).

In our greenhouse experiment, the treatments affected by isolates of *T. flavus* had significantly greater numbers of healthy seedlings in the case of soil artificially inoculation with Rhizoctonia or a combination of pathogenic factors compared to the inoculation with Fusarium, while a significant increase was observed in the number of healthy seedlings in soil artificially inoculated with Fusarium compared to the soil inoculated with Rhizoctonia or combination of pathogenic factors for treatments affected by isolates of *T. harzianum*. Therefore, it can be concluded that the effect of *T. flavus* on the reduction of sugar beet damping-off disease caused by *R. solani* or combination of pathogenic factors was higher than the case in which Fusarium was the only cause of this disease. However, the maximum effect of *T. harzianum* on the reduction of sugar beet damping-off disease occurred when its only cause was Fusarium. In this regard, the results of previous study by Nicoletti *et al*. (2009) also indicated that the effect of *T. flavus* on the inhibition of the growth *R. solani* was higher than other fungal pathogen of seedling damping off.

Furthermore, the results of our field studies in Karaj area indicated that the treatment, affected by isolate of *T. flavus* (T.F.K.3) had the maximum yield compared with other treatments and it had no significant difference with the treatment affected by isolate *T. harzianum* (T.H.K.1) (Table 4). According to the results of previous studies by Moayedi and Mostowfizadeh-Ghalamfarsa (2010) in the sugar beet field, the population of soil microorganisms after application of fungal antagonist, isolates of *T. harzianum* and *T. flavus* during the first crop year of this research led to the breeding population of these isolates especially *T. flavus*, so that they could maintain the effects of metabolites until the end of growth period.

Furthermore, according to the greenhouse and field experiments in our study, the difference between the antagonistic isolates of *T. flavus* or *T. harzianum* in terms of their capability to reduce the seedling damping-off disease can be caused by a wide range of activities and mechanisms including the production and secretion of different metabolites by these isolates which may be due to their genetic variation (Madi *et al.*, 1992).

The results of the present study may have practical use in the promotion of the health and growth of sugar beet in the field conditions through a non-chemical and ecological friendly strategy which can result in the cultivation of healthy products and the protection of the agricultural environment and biological resources.

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APPENDICES

Table 1: Effects of Antagonistic Isolates on Sugar Beet Damping-off Disease in the Greenhouse Experiment

Treatment	Healthy Seedling Number		
	15 Days	30 Days	45 Days
Untreated Control	10.00a*	10.00a	10.00a
Control Containing *R. solani* (R.S.K.4)	5.5l	6.00n	6.00j
Control Containing *R. solani* (R.S.K.5)	6.00k	6.25m	6.50i
Control Containing *F. proliferatum* (F.S.K.1)	7.00h	7.00j	8.00fg
Control Containing R.S.K.4, R.S.K.5 & F.S.K.1	5.5l	5.75o	6.00j
Carboxin-thiram+ R.S.K.4	6.50j	6.5l	7.00h
Carboxin-thiram+ R.S.K.5	6.75i	6.75k	7.00h
Carboxin-thiram+ F.S.K.1	7.75e	8.00f	8.75cd
Carboxin-thiram+ R.S.K.4, R.S.K.5 & F.S.K.1	6.00k	6.5l	7.00h
T.F.K.1+ R.S.K.4	8.50b	9.00b	9.00c
T.F.K.1+ R.S.K.5	8.50b	8.50d	9.00c
T.F.K.1+ F.S.K.1	8.00d	8.00f	9.00c
T.F.K.1+ R.S.K.4, R.S.K.5 & F.S.K.1	8.50b	9.00b	9.00c
T.F.K.2+ R.S.K.4	7.50f	8.00f	8.00fg
T.F.K.2+ R.S.K.5	7.50f	7.50h	8.00fg
T.F.K.2+ F.S.K.1	7.50f	7.50h	8.25ef
T.F.K.2+ R.S.K.4, R.S.K.5 & F.S.K.1	7.75e	8.50d	8.50de
T.F.K.3+ R.S.K.4	8.50b	9.00b	9.00c
T.F.K.3+ R.S.K.5	8.50b	8.50d	9.00c
T.F.K.3+ F.S.K.1	8.00d	8.00f	9.00c
T.F.K.3+ R.S.K.4, R.S.K.5 & F.S.K.1	8.50b	9.00b	9.00c
T.H.K.1+ R.S.K.4	7.50f	8.50d	8.50de
T.H.K.1+ R.S.K.5	7.50f	7.50h	8.00fg
T.H.K.1+ F.S.K.1	8.50b	8.75c	9.50b
T.H.K.1+ R.S.K.4, R.S.K.5 & F.S.K.1	7.50f	8.00f	8.25ef
T.H.K.2+ R.S.K.4	7.75e	8.50d	8.75cd
T.H.K.2+ R.S.K.5	7.50f	7.75g	8.25ef
T.H.K.2+ F.S.K.1	8.50b	9.00b	9.50b
T.H.K.2+ R.S.K.4, R.S.K.5 & F.S.K.1	7.50f	8.00f	8.25ef
T.H.K.3+ R.S.K.4	7.00h	8.00f	8.00fg
T.H.K.3+ R.S.K.5	7.25g	7.25i	7.75g
T.H.K.3+ F.S.K.1	8.25c	8.25e	8.50de
T.H.K.3+ R.S.K.4, R.S.K.5 & F.S.K.1	7.00h	7.50h	8.00fg

*Values marked with the same letter (s) in the columns are not statistically different according to Duncan's Multiple Range Test ($p > 0.01$).

Table 2: Effects of Antagonistic Isolates of *Talaromyces flavus* and *Trichoderma harzianum* on Sugar Beet Damping-off Disease and Total Yield of Sugar Beet in Karaj Field Experiment during the Year 2008

Treatment	Healthy Seedling Number (Stand)				Total Yield Mean (kg/ha)
	20 Days After Planting	30 Days After Planting	40 Days After Planting	60 Days After Planting	
T.F.K.1	286.00b	297.00d	111.25a	108.75b	42083.00abc
T.F.K.3	364.00a	379.75c	122.00a	125.25a	44167.00abc
T.H.K.1	400.00a	414.50b	113.50a	120.00ab	54167.00a
T.H.K.2	376.75a	435.50a	116.25a	119.00ab	47917.00ab
Carboxin-thiram	269.50a	271.75e	86.75a	87.25c	37292.00bc
Control	225.25a	208.75f	84.00b	82.25c	31042.00c

*Values marked with the same letter (s) in the columns are not statistically different according to Duncan's Multiple Range Test ($p > 0.01$).

Table 3: Effects of Antagonistic Fungal Isolates of *Talaromyces flavus* and *Trichoderma harzianum* on Sugar Beet Damping-off Disease and Total Yield of Sugar Beet in Karaj Field during the Year 2009

Treatment	Healthy Seedling Number (Stand)				Total Yield Mean (kg/ha)
	20 Days After Planting	30 Days After Planting	40 Days After Planting	60 Days After Planting	
T.F.K.1	141.75b	148.50b	145.50b	145.50b	60104.00a
T.F.K.3	184.00a	185.50a	186.00a	185.50a	63750.00a
T.H.K.1	97.75c	147.00b	147.50b	147.25b	59375.00a
T.H.K.2	80.75c	80.75ca	81.25c	80.50c	36146.00b
Carboxin-thiram	89.00c	88.50ce	75.75c	72.25cd	32188.00b
Control	49.50d	48.75d	51.50c	50.50d	26667.00b

*Values marked with the same letter (s) in the columns are not statistically different according to Duncan's Multiple Range Test ($p > 0.01$).

Table 4: Statistical Grouping of the Treatments Applied in Field Studies during the Years 2008 and 2009 at Different Time Intervals in Terms of Average Number of Healthy Seedlings and Total Yield Obtained by Combined Analysis

Treatment	Healthy Seedling Number (Stand)				Total Yield Mean (kg/ha)
	20 Days After Planting	30 Days After Planting	40 Days After Planting	60 Days After Planting	
T.F.K.1	213.88bc	222.88c	128.38b	127.13b	51093.50c
T.F.K.3	274.00a	282.63a	154.00a	155.38a	53958.50b
T.H.K.1	248.88ab	280.75a	130.500b	133.63b	56771.00a
T.H.K.2	228.75b	258.13b	98.75c	99.75c	42031.00d
Carboxin-thiram	179.25c	180.13d	81.25d	79.75d	34740.00e
Control	137.38d	128.75e	67.75e	66.38e	28854.00f

*Values marked with the same letter (s) in the columns are not statistically different according to Duncan's Multiple Range Test ($p > 0.01$)